

AD-A054 433

WASHINGTON UNIV ST LOUIS MO
CONTROLLED RELEASE OF DRUGS FOR TREATMENT AND PROPHYLAXIS IN SC--ETC(U)
OCT 76 R E SPARKS

F/G 6/15

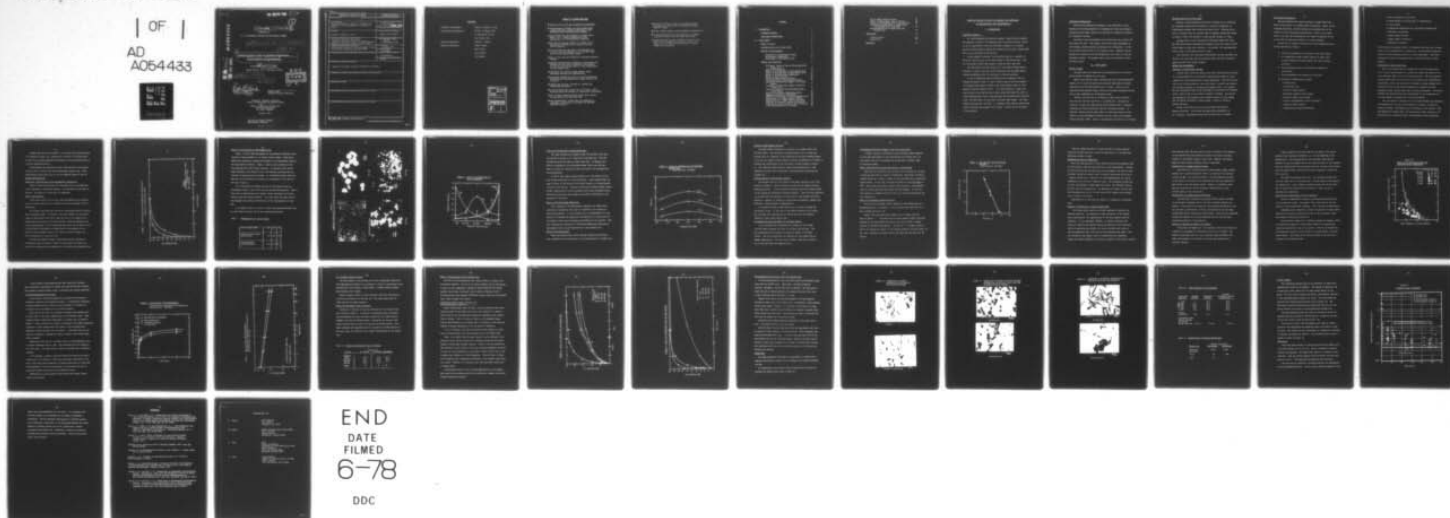
DAMD17-75-C-5016

NL

UNCLASSIFIED

| OF |

AD
A054433



FOR FURTHER TRAN

AD A 054433

AD No. JDC FILE COPY

①
⑨ Final Report, 1 Oct 74-7 Aug 76,
submitted to

U. S. Army Medical Research and Development Command

⑩ Robert E. Sparks / ⑪ 42p.

⑫ Oct 76

⑬
CONTROLLED RELEASE OF DRUGS FOR TREATMENT AND PROPHYLAXIS
IN SCHISTOSOMIASIS AND TRYPANOSOMIASIS

⑭ Contract No. DAMD17-75-C-5016

(Report Period: Oct. 1, 1974-Aug. 1, 1976)

⑮ 3A762759A831

⑯ PP

DDC
RECEIVED
MAY 30 1978
B

Robert E. Sparks
Robert E. Sparks
Principal Investigator

Blanche Jones
Sponsored Projects Associate

Biological Transport Laboratory
School of Engineering and Applied Science
and
Office of Laboratory Animal Care
Washington University
St. Louis, Missouri

October, 1976

Approved for public release;
distribution unlimited

374 444

JOB

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Controlled Release of Drugs for Treatment and Prophylaxis in Schistosomiasis and Trypanosomiasis		5. TYPE OF REPORT & PERIOD COVERED Final Report 1 Oct. 1974 - 1 AUG. 1976
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Robert E. Sparks, D. Eng.		8. CONTRACT OR GRANT NUMBER(s) DAMD 17-75-C-5016 ^{ne}
9. PERFORMING ORGANIZATION NAME AND ADDRESS Biological Transport Laboratory Washington University School of Engineering and Applied Science, St. Louis, Missouri 63130		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62759A 3A762759A831.00.097
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick, Frederick, Maryland 21701		12. REPORT DATE October 1976
		13. NUMBER OF PAGES 43 pages
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)		

PERSONNEL

Principal Investigator:	Robert E. Sparks, D. Eng.
Associated Investigators:	Norbert S. Mason, Ph.D.
	E. Kaye Smith, D.V.M.
	Curt Thies, Ph.D.
Research Technician:	Timothy C. Finley
Research Assistants:	Darryl Jacobs
	Mark Prince
	Gary Cupples
	John Bogdan

ACCESSION for	
NTIS	White Section <input checked="" type="checkbox"/>
DDC	Buff Section <input type="checkbox"/>
UNANNOUNCED	<input type="checkbox"/>
JUSTIFICATION	
BY	
DISTRIBUTION/AVAILABILITY CODES	
Dist. AVAIL. and/or SPECIAL	
A	

SUMMARY OF ACCOMPLISHED WORK

- Poly(dl lactic acid) was successfully synthesized.
- Microcapsules of Astiban acid were formed using a phase separation technique developed in this laboratory under previous project support.
- Drug release rate was measured in a shaker-water bath at 37°C. Results compared well with measurements in a rotating-bottle extractor.
- The time of solution of half of a sample of non-encapsulated Astiban acid was found to be less than two minutes.
- At an optimum stirring speed our method gives up to 60% of the microcapsules in the 180-300 μ range used for most drug-release studies.
- This is also near the optimum for obtaining sustained release.
- Changing solidification temperature, hydrocarbon non-solvent type, and rate of addition of non-solvent did not have appreciable effect on size distribution or drug release rate.
- Increasing the volume of phase inducer caused additional problems with agglomeration.
- Increasing polymer/drug ratio to yield microcapsules containing 50% drug raised half release times to 20-30 hours.
- Compressing the drug, followed by crushing and sieving was beneficial.
- A new procedure gave a half time of 16 hours. This procedure should be amenable to considerable improvement.
- Use of higher density solvents gives better control in the process with this dense drug.
- Also encapsulated, to show that the technique is applicable, were Astiban, niridazole and antimony potassium tartrate.

- Modified technique of making microcapsules using microwax gave a better size distribution and more spherical shape.
- Using a higher density solvent minimized agglomeration.
- Decreasing initial PLA concentration gave a higher fraction of particles in the small size range.
- Studied the drug release in hamsters using the best batch of microcapsules and injection in rear leg. Blood level vs. time and distribution of drug in organs were determined.

CONTENTS

I. INTRODUCTION	1
CONTROLLED RELEASE	1
INJECTABLE MICROCAPSULES	2
II. FINAL REPORT	2
CHOICE OF DRUGS	2
RATIONALIZATION OF IN VITRO TESTS	3
METHODS AND MEASUREMENTS	3
Synthesis of Bioabsorbable Polymer	3
Microcapsule Preparation	4
Measurement of Drug Release Rate	5
RESULTS AND DISCUSSION	6
Base-line: Solution Rate of Non-encapsulated Astiban Acid	6
Size of Microcapsules	6
Effect of Stirring Rate on Microcapsule Size	8
Effect of Stirring Rate on Drug Release Rate	11
Effect of Solidification Temperature	11
Nature of the Non-solvent	11
Volume of Phase-Inducer Solution	13
Controlling Rate of Non-Solvent Addition	13
Increasing the Molecular Weight of the Phase Inducer	13
Increasing the Molecular Weight of the Poly (Lactic acid)	14
Effect of Drying the Drug and Maintaining a Closed System	14
Effect of Increasing Polymer/Drug Ratio	14
Agglomerating Drug by Compression	16
Addition of Surfactant to Lessen Agglomeration	16
Substitution of a Higher Density Solvent	17
Substitution of a Higher Density Nonsolvent	17
Addition of Microwax to Harden the Capsules	17
Lower Initial Concentration of PLA in Solution	18
A New Microencapsulation Procedure	20

Use of Higher Density Solvent	23
Effect of Microcapsule Size on Payload	23
Effect of Microcapsule Size on Release Rate	24
Differences Between Tests in Shaker Bath and Rotating Bottle Apparatus	24
Microencapsulation of Drugs other than Astiban Acid	27
CONCLUSIONS	27
In-Vivo Studies	32
Observation	32
REFERENCES	35

CONTROLLED RELEASE OF DRUGS FOR TREATMENT AND PROPHYLAXIS
IN SCHISTOSOMIASIS AND TRYPANOSOMIASIS

I. INTRODUCTION

CONTROLLED RELEASE

In a well-developed host-parasite system, a drug effective against the parasite is likely to be somewhat toxic to the host as well. Hence, it is not surprising to find that preferred treatment of a tropical parasite disease involves giving the curative dose in small, spaced doses which can be tolerated by the patient.

A good example is Astiban, the preferred drug for *S. mansoni* infections, which is given in 5-7 spaced doses of 400-500 mg each. Even so, the patient usually shows signs of toxicity after each dose.

The side effects and the logistics of such spaced therapy make it difficult to effect cure under field conditions, and also make spaced therapy inconsistent with the realities of military medicine.

A key to eliminating these problems is the recognition that the minimum therapeutic level for some of these drugs is very low compared to the peak concentration reached in the blood shortly after an intramuscular injection (Sparks, 1973). If it were possible to supply the drug in such a form that the blood level stayed well below the level of toxic manifestations, while remaining above the therapeutically effective level, the same amount of drug would last many times longer. The ideal dosage form would be a one-shot i.m., giving no side effects, which would stay in the body long enough to be curative. This is one of the goals of this project.

INJECTABLE MICROCAPSULES

Utilizing the experience developed in this laboratory, we are studying the microencapsulation of representative drugs into polymer-covered particles small enough to be slurried in a medium and injected by a hypodermic syringe.

The walls of the microcapsules will control the rate at which the drug enters the body. The polymer used will be slowly degradable by body enzymes, giving innocuous end-products for elimination. In this way, no debris will be left at the injection site. Examples of such polymers are polylactic acid and polyglycolic acid, which is used for resorbable sutures. The polymer used so far in this project is poly (dl-lactic acid).

II. FINAL REPORT

CHOICE OF DRUGS

Although almost all drugs may be microencapsulated, the process generally must be adapted for each drug.

Highly water soluble drugs, although they may be microencapsulated readily, do not give prolonged release because high osmotic pressure draws water into the microcapsule until it bursts. Hence, in considering antischistosomal drugs, Astiban and antimony potassium tartrate are not good candidates for developmental work.

However, the acid form of Astiban has a much lower solubility than the salt form (0.5 gm/100 ml vs 30 gm/100 ml). From past experience, we know that drugs having this solubility have a reasonable likelihood of being successfully used for controlled release. In addition, Astiban acid has been shown to be much more effective than Astiban in mice and hamsters (Stohler and Frey, 1964) and in monkeys (Bruce and Sadun, 1966). Hence it was chosen as the focus of our efforts.

RATIONALIZATION OF IN VITRO TESTS

Recently a phase-separation method was developed in our laboratory for preparing long-acting microcapsules of narcotic antagonists in bioabsorbable polymer walls (Cicero and Thies, 1974). Rotating bottle tests of release rates for capsules $<300\mu$ in diameter showed 50% release of drug in 2 days, 75% release in 4 days and 90% release in 7 days.

In vivo tests in rats (subcutaneous injection) showed that there was 100% effectiveness (antagonism) exceeding 7 days and a mean of 61.4% effectiveness 14 days after injection. By contrast, the unencapsulated drug is only 2 to 4% effective after 7 days.

This indicates that injectable microcapsules can give extended drug release, and also shows that microcapsules which show slow release in vitro also give slow release in vivo.

METHODS AND MEASUREMENTS

Synthesis of Bioabsorbable Polymer

Poly(dl-lactic acid) was chosen as the wall-forming material because it is bioabsorbable and its solubility characteristics make it a good candidate for microencapsulation by phase separation. With 85% dl-lactic acid as the starting material, the cyclic dimer or lactide was prepared and purified according to the method of Kulkarni (1971). The lactide was then polymerized under vacuum according to the procedure of Schneider (1951), using 1% ZnO as the catalyst instead of litharge. The ZnO was then removed by centrifugation from a benzene solution of the polymer and the polymer recovered by freeze drying. A batch of 230 gm of polymer was made.

100 gm of poly(dl-lactic acid) was obtained from Southern Research Institute. This polymer was made using tetraphenyl tin as a catalyst. Microcapsules were made from both lots of polymer.

Microcapsule Preparation

The microencapsulation method developed by Lapka, Mason and Thies is the subject of a pending patent application. Hence, in the following discussion, specific amounts and techniques are not described to avoid jeopardizing patentability. Every care is being taken to see that the technology will be readily licensable for use in the field of long-term treatment of parasite diseases.

The process of forming microcapsules by phase separation may be briefly described as follows:

1. The drug is dispersed in a solution of the wall polymer.
2. An incompatible polymer (phase inducer) is added until a second polymer-rich phase appears and coats the drug particles.
3. The microcapsule walls are solidified by addition of non-solvent.
4. The microcapsules are recovered by filtration.

The following variables were surveyed:

- solvent type
- non-solvent type
- nature of phase inducer
- molecular weight of wall polymer
- ratio of drug to wall polymer
- initial concentration of pLA in solution
- amount of phase inducer
- temperature during solidification

- rate of addition of non-solvent
- vacuum drying of the drug prior to encapsulation
- stirrer speed
- compression agglomeration of drug before encapsulation.
- addition of surfactant
- higher density solvent
- higher density nonsolvent
- addition of microwax.

Each variable was examined briefly to determine which ones had a strong effect on size distribution or release rate. More detailed studies will be made of those variables which are of major importance. Good microcapsules will result from applying what we learn from these variable studies.

Measurement of Drug Release Rate

Most of the release data were generated in the following manner: 10 to 15 mg of microcapsules of a single size range were placed in 100 ml of deionized water in a 125 ml screw-cap bottle. This was placed in a water shaker bath at 37°C set for 1 1/8" strokes at 80 cycles/minute. Periodically, about 4 ml were filtered through a syringe equipped with a millipore filter and the filtrate analyzed for antimony by atomic absorption using a Perkin Elmer Model 503 spectrophotometer. The percent released was calculated from the concentration in the supernatant and the total amount of Astiban acid in the microcapsules.

The total amount of Astiban acid in the microcapsules was determined by dissolving 10 to 15 mg of microcapsules in acetone, in which PLA dissolves rapidly, then adding water and boiling off the acetone. When the temperature reached 100°C, the solution was cooled, diluted to the 100 ml mark in a volumetric flask, and analyzed by atomic absorption.

Release data were also obtained in a rotating bottle apparatus made by E. Menold of Lester, Pa., operating at 40 rpm in a 37°C water bath. In this case 15 mg microcapsules were placed in 75 ml deionized water in a 100 ml cylindrical bottle.

Microcapsules were observed with the light microscope during manufacture, after isolation, and after determining release rates. These observations provide many clues to why the capsules behave as they do.

RESULTS AND DISCUSSION

Base-line: Solution Rate of Non-encapsulated Astiban Acid

Figure 1 shows the solution rate of Astiban acid, as received and after compression, crushing and sieving. The half-time is less than two minutes. This gives us a base-line for comparison.

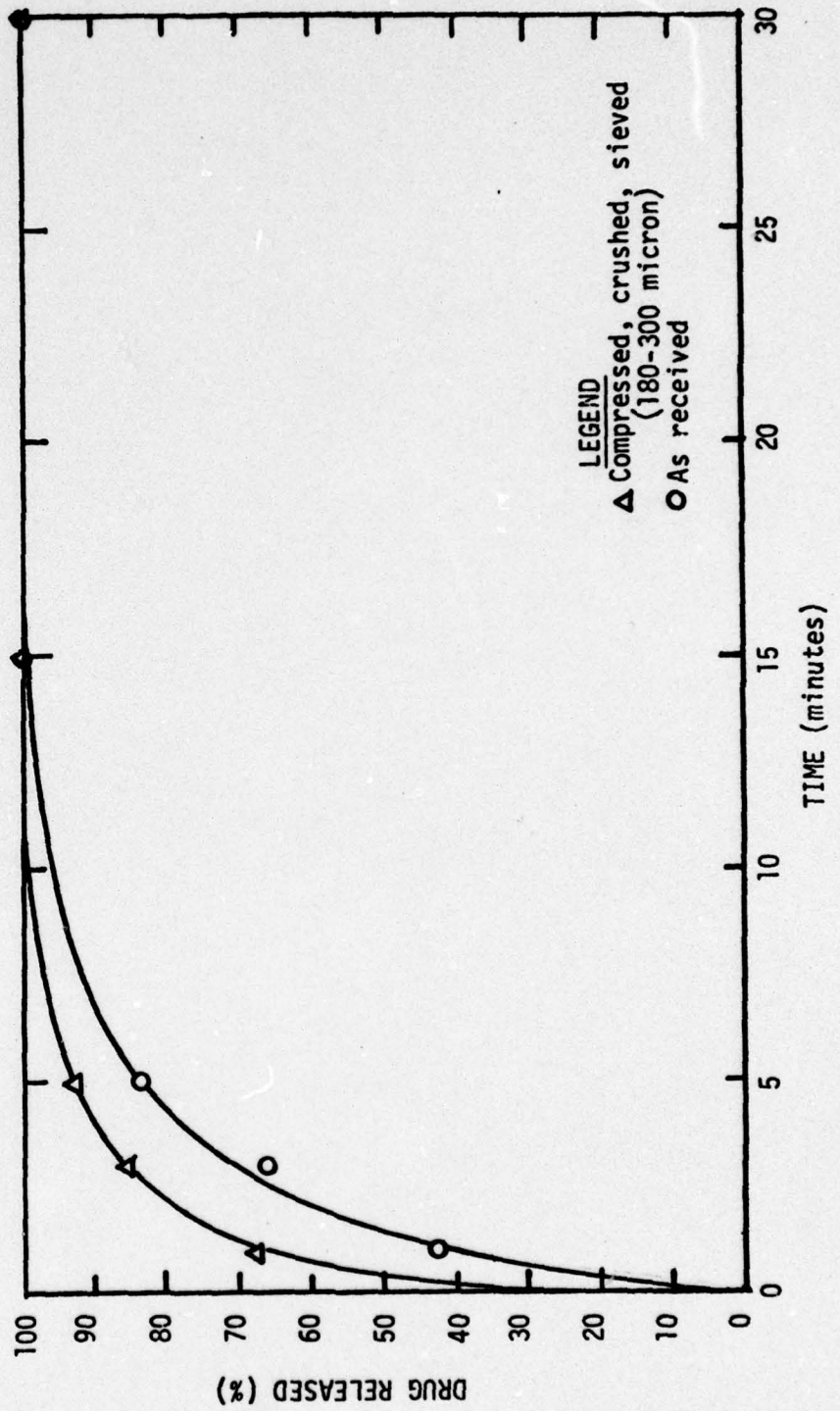
Size of Microcapsules

After drying in air for two days, the microcapsules were separated into size ranges using a set of standard sieves with openings of 106 to 600 microns.

Our studies have shown that 300 micron microcapsules may be injected with an 18 gauge needle. In general, the inside diameter of the needle cannot be much smaller than three times the size of the largest sieve opening which will pass all the microcapsules, otherwise binding occurs in the syringe needle. We are specifying 300 microns as the upper cut-off for injectable microcapsules, although smaller microcapsules are preferred to obtain similar release (The effect of size on release rate will be discussed in a later section).

The fraction passing through a 300-micron sieve but retained by a 180-micron sieve was used to compare the runs except for those runs in which the release rate was measured as a function of microcapsule size.

FIGURE 1. SOLUTION RATE OF NON-ENCAPSULATED ASTIBAN ACID
(Measured on Shaker-Water Bath)



Effect of Stirring Rate on Microcapsule Size

Figure 2 shows light micrographs of representative embryonic microcapsules of drug obtained at 3 different stirrer speeds. These micrographs were obtained by cooling the droplets at an intermediate stage of the microcapsule formation. Figure 3 shows a sieve analysis of the microcapsules which were finally isolated. As expected, when stirrer speed increases, the fraction of very fine droplets increases and the fraction of large particles decreases. At intermediate shear rates the mean size of the particles is observed as a maximum, as is the case for 160 rpm in Figure 3.

It is interesting to compare the size of the droplets from the intermediate stage to that of the finally isolated microcapsules. Table I shows that as RPM increases the largest droplet decreases and the size fraction above 300 micron decreases. The table shows also that there is some agglomeration during solidification of the microcapsules for each RPM.

It is apparent that it is easy to control the mean microcapsule size by controlling the shear rate in the coacervating solution.

Table I. Agglomeration vs. Stirrer Speed

Stirrer Speed (RPM)	224	160	112
Largest frozen droplet seen (micron)	52	112	250
% microcapsules above 300 micron	4.8	40.1	79.1

Figure 2
EMBRYONIC MICROCAPSULES OF ASTIBAN ACID IN POLYLACTIC ACID

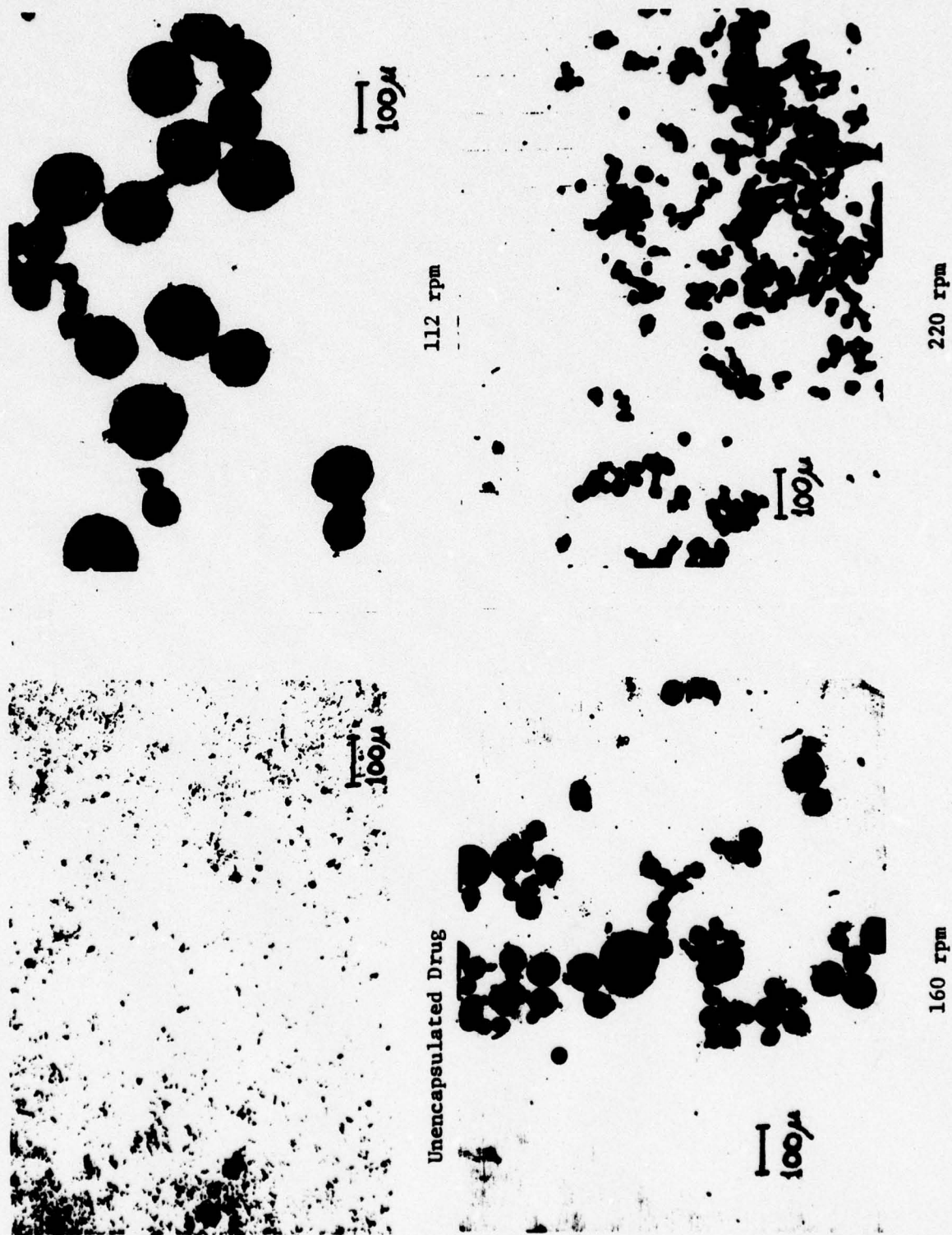
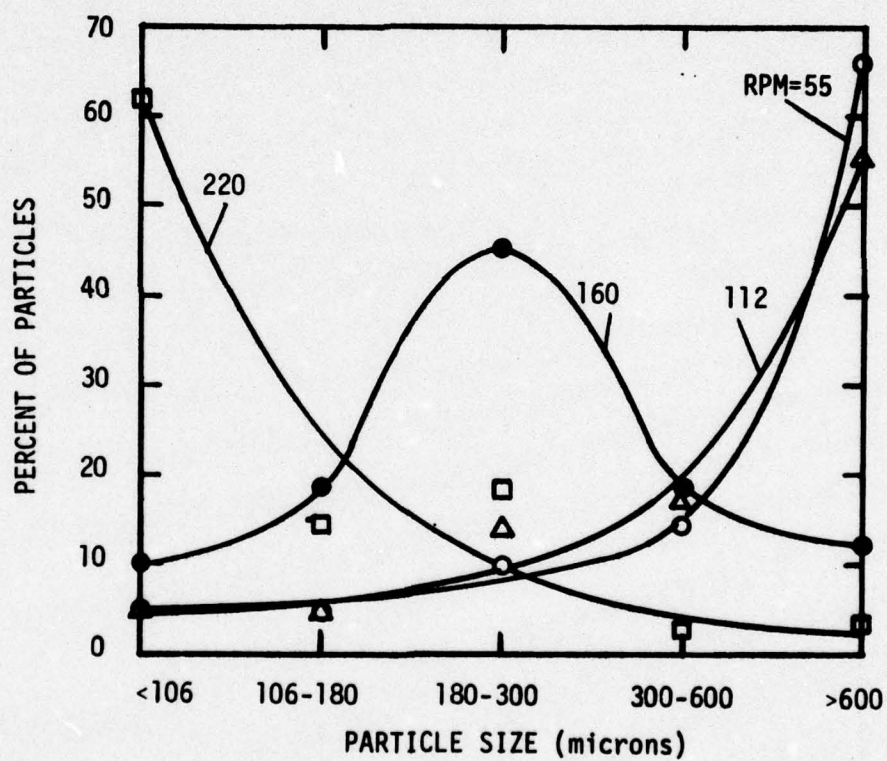


FIGURE 3. EFFECT OF STIRRING RATE ON
SIZE DISTRIBUTION



Effect of stirring Rate on Drug Release Rate

The times required for release of 50%, 75% and 90% of the drug are plotted in Figure 4, as a function of stirring rate. Only the 180-300 μ fraction was used to obtain these data. It appears that there is a maximum in the controlled-release effect near 160 rpm, where 2.6 hours are required for 90% of the drug to be released from the microcapsules.

It may be that higher speeds probably cause more defects in the wall through collisions of the microcapsules. Lower speeds permit the drug to settle to the bottom of the vessel because of its high density relative to the solvent. This also permits more droplet-droplet contact. Further examinations of the microcapsule walls using scanning electron microscopy should give some insight into possible differences in the integrity of the walls.

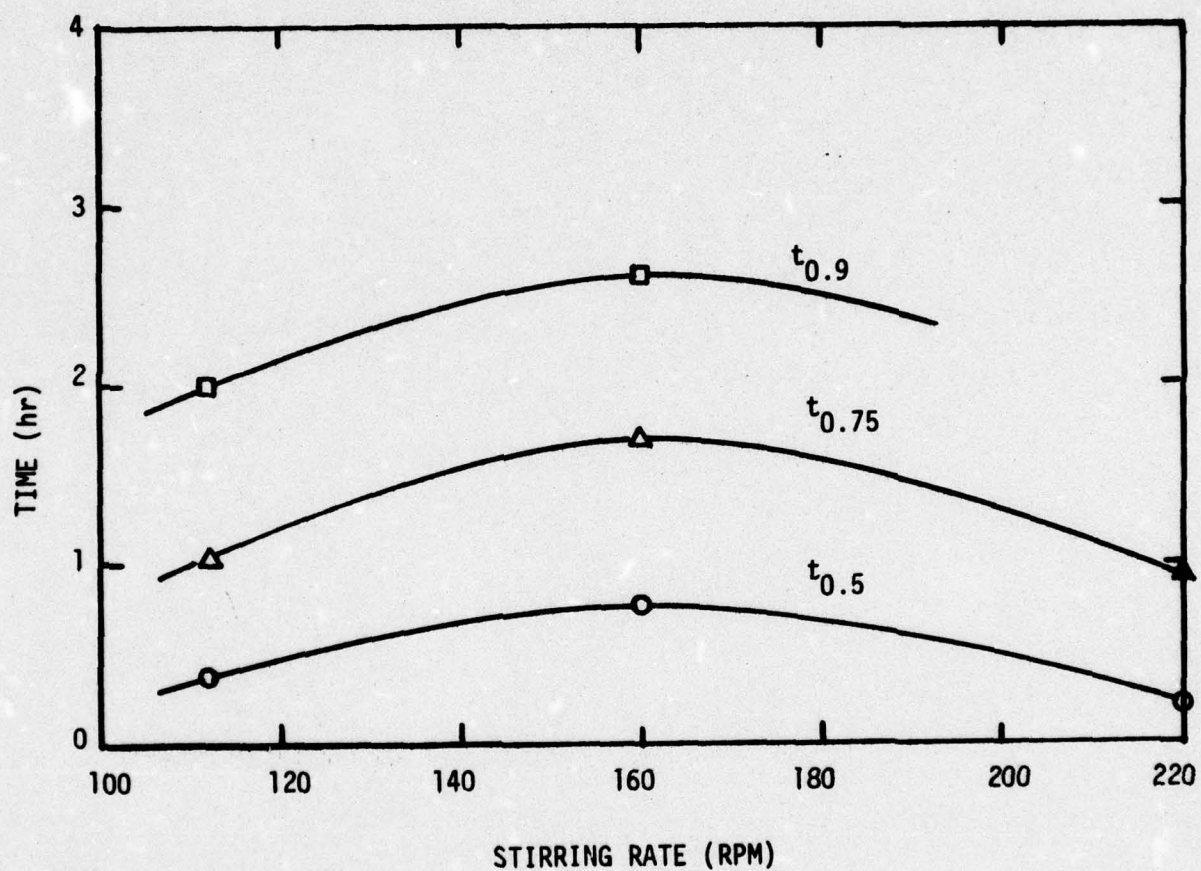
Effect of Solidification Temperature

After droplets of the drug-polymer suspension are formed under conditions of agitation, they must be solidified to be isolated as usable microcapsules. In our procedure this is accomplished by a combination of lowering the temperature and addition of a non-solvent for the polymer, which causes shrinkage and hardening of the capsules. When this technique was carried out at differing temperatures there was no appreciable effect on size distribution or drug release rate.

Nature of the Non-solvent

Three non-solvents were tested: pentane, heptane and isooctane. This variation had no strong effect on size distribution or release rate.

FIGURE 4. EFFECT OF STIRRING RATE ON FRACTIONAL
RELEASE TIME
(Size Fraction: 180-300 μ)



Volume of Phase-Inducer Solution

The phase-inducer solution is a solution of a polymer other than the wall-former. The function of the phase-inducer is to provide the driving force for formation of the coacervate of the wall-forming polymer. In one run the volume of phase-inducer solution was doubled in an attempt to increase the insolubility of the coacervate, perhaps helping to harden it. This additional phase-inducer caused the microcapsules to clump together so solidly that the run was terminated without evaluating the release rate.

Controlling Rate of Non-Solvent Addition

The Astiban acid is in the form of very small particles, only a few microns in diameter. They are wetted very well by the polymer solution, dispersing readily. In the coacervate formation step, they become trapped in the forming and coalescing coacervate droplets. Since the drug particles are so small and the coacervate is fairly fluid, the droplets are nearly spherical. However, on addition of non-solvent the geometry changes from spherical, largely because of agglomeration.

An attempt was made to gain control over the step by adding the non-solvent slowly. In two runs it was added carefully at 0.42 ml/min. The resulting size distribution and release rate were not markedly superior to those from previous runs.

Increasing the Molecular Weight of the Phase Inducer

It was reasoned that increasing the viscosity of the polymer solution should decrease the rates of collision and settling. This was accomplished by increasing the molecular weight of the phase inducer. The size distribution was improved but the release did not change significantly. This was run at 96 RPM. There was no control run at the same speed and temperature yet.

Increasing the Molecular Weight of the Poly(Lactic acid)

A slight increase in poly(lactic acid) molecular weight appeared to give some improvement in size distribution and release rate, but this effect can only be determined by working with a broader range of molecular weights.

Effect of Drying the Drug and Maintaining a Closed System

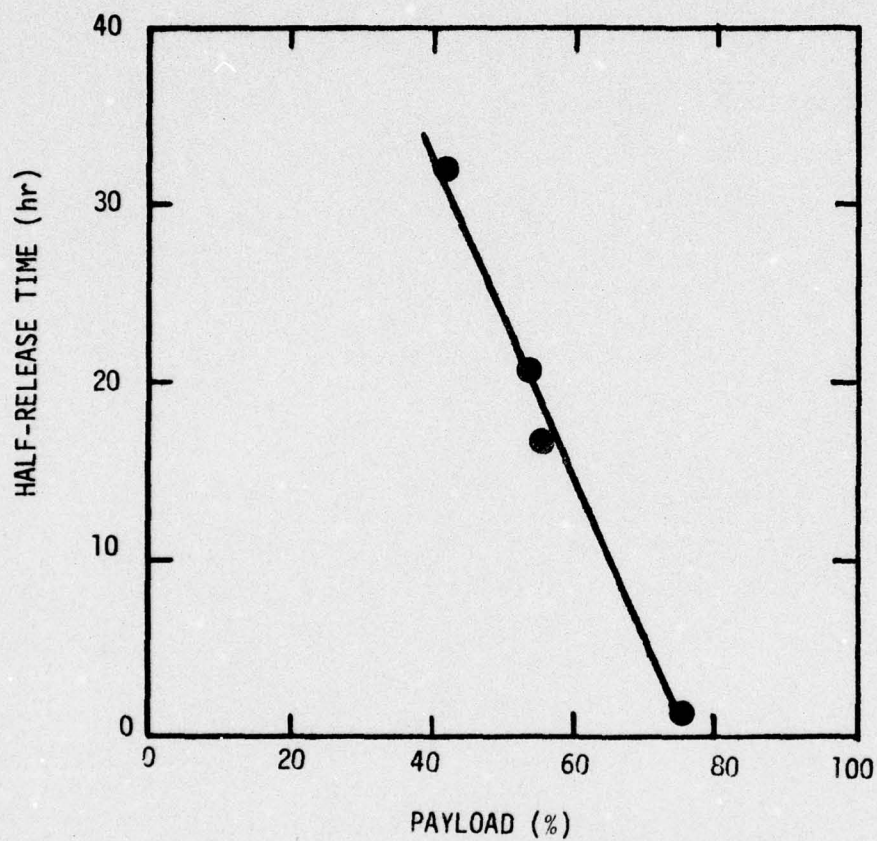
There was the possibility that moisture on the surface of the drug or moisture entering the system by condensation could affect the microcapsule properties. Hence, a number of runs were made with a completely closed system. In one case, the drug was dried for 16 hrs in vacuo at 40°C. Both these runs showed a rather large fraction of microcapsules over 600 microns and short half-times for drug release. It could be that moisture is somewhat beneficial both for the size distribution and the release rate.

Effect of Increasing Polymer/Drug Ratio

The drug can be somewhat better imbedded in the polymer when the polymer/drug ratio is increased. Up to this point the ratio was 1/3 to give a design payload of 75%.

Several runs were made with a higher ratio to obtain the data shown in Figure 5 . The proportion of large capsules (>600 μ) increased markedly and stirring speed was raised in one run to obtain a higher fraction of 180-300 μ microcapsules. The most dramatic change, however, was in the release rate which, in one run, was slowed to the point where 30 hrs. were required for release of half the drug, and 138 hours for 75% release.

FIGURE 5. HALF-RELEASE TIME FOR VARIOUS
PAYLOADS
(Size fraction: 180-300 μ)



With our present procedure it seems possible to obtain capsules which will release drug over a period of many hours or a few days when sufficient polymer is used.

Agglomerating Drug by Compression

Since the drug particles are much smaller than the microcapsules made by this process, there are many particles in each microcapsule. Ideally, if there were only one drug particle per microcapsule, the area for drug diffusion through the polymer would be greatly reduced and would lead to a reduced release rate. Therefore, we tried agglomerating the drug by compression in a die with a hydraulic press. The resulting cylinder was crushed, recompressed, crushed again and sieved. The 180-300 μ fraction was then used for encapsulation. By comparison to control runs the half time increased from 0.76 to 1.0 hour and the 75% release time increased from 1.7 hours to 2.1 hours.

Compression of the dry drug was superior to comparison of moistened drug.

Addition of Surfactant to Lessen Agglomeration

The surface active agents are usually employed to increase the emulsion stability. By choosing the right surfactant for the system under consideration, the agglomeration of the microcapsules could be minimized. In our experiment "ARLACEL", a nonionic surfactant was chosen to stabilize the emulsion during the addition of the nonsolvent. After the coacervate was formed, the reactor contents were cooled to the low temperature and a few drops of the surfactant were added. This was followed by the regular cycles of washing with the nonsolvent. During the dropwise addition of surfactant, samples of the reactor contents

were observed under the microscope to check the effect of the addition. It was observed that this addition resulted in the loss of wetting property of the polymer forming a pasty mass. However, this method might be useful using a different class of surfactant.

Substitution of a Higher Density Solvent

The search for a neutrally buoyant system using a higher density solvent led to tetra-bromo-ethane (TBE) or a mixture of the previous solvent and TBE. The original problem of the microcapsules settling to the bottom of the reactor was eliminated and they were observed to move freely in the new solvent system. However, a difficulty arose when the contents were cooled to subzero temperature due to the solidification of this new solvent.

Substitution of a Higher Density Nonsolvent

The same need to maintain a neutrally buoyant system, extended to the process of washing, led to the trial of heavier density non-solvent. Freon (Tf)113 was chosen for this substitution because it met the two basic qualifications of being a nonsolvent for pLA and being considerably heavier than previous nonsolvents. The batch was terminated because the addition of the freon separated the pLA from the drug, preventing formation of microcapsules.

Addition of Microwax to Harden the Capsules

The quality and sphericity of the capsules could be increased considerably by hardening the coacervate as soon as it is formed. One method of achieving this is to use a microwax, which disperses into small microcapsules (of the order of microns) when dispersed in a suitable "solvent".

A small quantity of microwax (100 mg) was added to the reactor contents after coacervate formation, i.e., at the beginning of the washing cycle. The wax dispersed in the continuous phase and was expected to coat the capsule surface to retain the shape of the capsules. After the capsules have been recovered and dried, the wax on the surface could be removed using a solvent which doesn't dissolve or affect the PLA in any way.

The method was somewhat effective. The resulting capsule size was very large; and it is believed that a certain amount of wax entered the capsule wall. Also, finding a suitable solvent for the wax which did not affect the polymer wall was difficult. Thus, the use of microwax was discontinued.

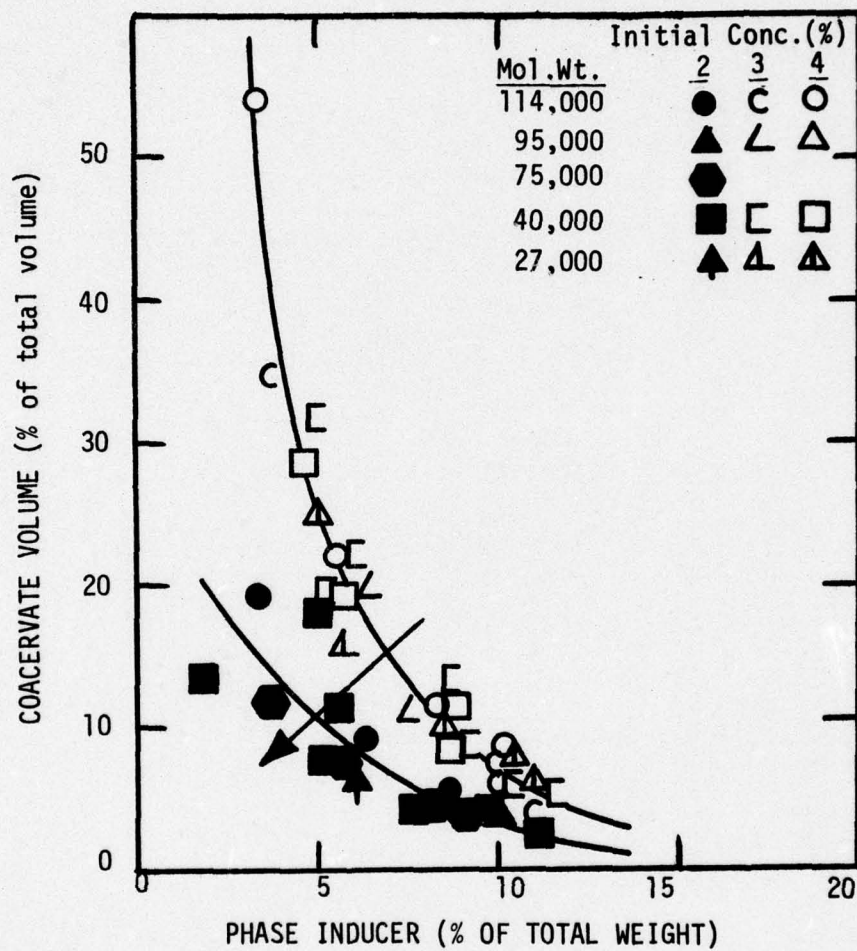
Lower Initial Concentration of PLA in Solution

Success in making hard, spherical microcapsules depends mainly on the coacervate volume. The smaller this volume the less are the chances of the microcapsules colliding. Thus, the shape is maintained.

Experiments were conducted to relate the quantity of phase inducer added and the initial concentration of pLA to the coacervate volume. The results are plotted in Figure 5-A.

The coacervate volume can be reduced by making two changes; either by reducing the quantity of phase inducer added or by lowering the initial concentration of pLA in the solvent. Both can be obtained by increasing the volume of solvent added for the same amount of pLA and phase inducer. Any change in the direction shown by the arrow will decrease the coacervate volume.

Figure 5-A
EFFECT OF PLA MOLECULAR WEIGHT AND
CONCENTRATION ON COACERVATION



A few batches of microcapsules were made using this technique and a significant improvement in capsule size distribution was obtained. The procedure requires further study to determine the optimum conditions.

A New Microencapsulation Procedure

At this point a new microencapsulation procedure was developed to overcome a problem in our standard procedure. A confidential disclosure of this procedure will be written for the Army when it is worked out in detail, and if it appears to be the best procedure.

After three trial runs, we succeeded in making a microcapsule batch in which 60% of the final capsules were in the 180-300 μ range. These capsules showed a significant improvement in release time, as shown in Figure 6, with a half-time of 16 hours as measured on the shaker apparatus. A portion of these capsules were also tested on the rotating bottle apparatus, giving the other curve in Figure 5. This shows that the two tests are consistent, although the absolute values of the release times differ somewhat.

These data do not plot as a straight line on a semi-logarithmic plot of percent remaining drug vs. time. This indicates that the release is not first order. The rapid initial release is probably due to imperfect capsules.

It is possible to predict long-term release from these data using a log-log plot, as in Figure 7, which gives excellent correlation of the data. This would allow estimation of plasma concentration at long times from knowledge of the rate of metabolism of the drug and the rate of pickup and release from tissues as concentration varies.

Modification of this procedure should permit much longer release times to be obtained.

FIGURE 6. DRUG RELEASED FROM MICROCAPSULES

(Encapsulated by Modified Phase Separation)
(Particle Size: 180-300 μ)

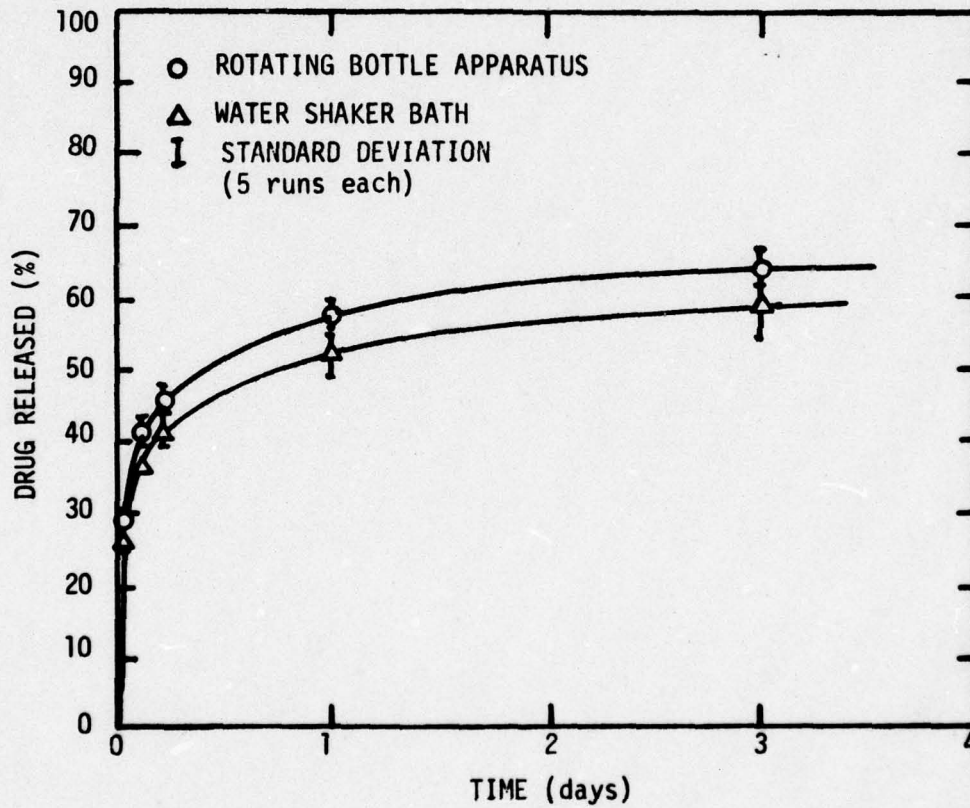
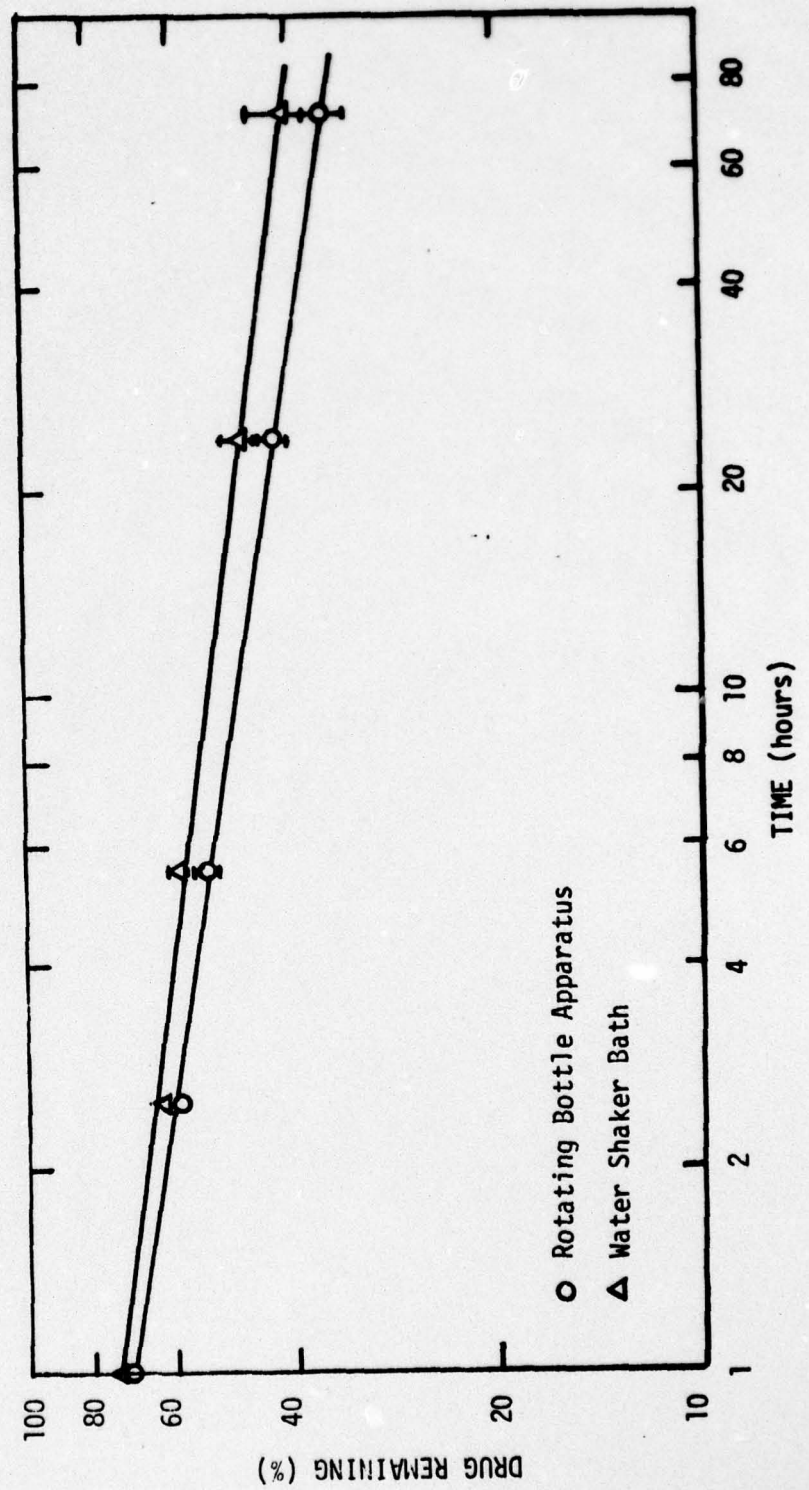


FIGURE 7. DRUG RELEASED FROM MICROCAPSULES
(Encapsulated by Modified Phase Separation)
(Particle Size: 180-300 μ)



Use of Higher Density Solvent

The high density of the Astiban acid causes considerable difficulty with agglomeration because of its tendency to settle to the bottom of the encapsulation vessel except at high speeds. A higher density solvent would minimize this problem.

Using a heavier solvent, we have obtained a good size distribution of particles working at 112 and 160 rpm. The drug release data for these runs are now being obtained.

Effect of Microcapsule Size on Payload

Payload as a function of size was determined for two typical runs, and is shown in Table II. In general, the payload tends to decrease with microcapsule size and the actual payload for all sieved sizes is somewhat less than the design payload. Apparently the fraction >600 micron fraction must be richer in drug than the design payload. This again indicates that agglomeration may be caused by the high density of the drug, since the particles with a higher drug payload are the larger ones.

Table II. Effect of Microcapsule Size on Payload

Run No.	Payload (%)		
	DJ 2/20/75	GC 6/20/75	PC 7/6/76
<u>Fraction</u>			
<106	55.9	41.0	55.8
106-180	68.7	51.5	61.3
180-300	68.1	53.3	64.2
300-600	69.2	62.3	-
Design	75	67	67

Effect of Microcapsule Size on Release Rate

The size of the microcapsules has a marked effect on release rate, as would be expected. For one of our early standard runs at 160 rpm, and a later run with compressed, crushed and sieved drug with 67% design payload, this effect of particle size is shown in Figures 8 and 9. As expected, the large capsules (300-600 μ) release slowly and the smallest ones (<106 μ) release very rapidly.

Differences Between Tests in Shaker Bath and Rotating Bottle Apparatus

Recent observations under the light microscope indicate that microcapsules tested in the shaker water bath stick together to a greater extent than in the rotating bottle apparatus, especially after several days of testing. This could have an effect on the somewhat longer release times measured in the shaker bath, in addition to the different degrees of mixing encountered in the two pieces of apparatus.

It is of interest to ask why the droplets stick together in either case, and then ask why they stick together more in the shaker bath.

First, it is likely that the small amount of water absorbed by the polylactic acid acts as a plasticizer, gradually softening the polymer enough to permit some sticking together. There is also the possibility that hydrolytic chain scission occurs upon long-term immersion in buffer. In order to study hydrolysis of the polymer, Kulkarni (1971) had to use strongly basic solution or liver homogenates. From his work, it would not be expected that a measurable degradation of the polymer would occur in 24 hours. However, it is possible that some hydrolysis could occur at longer times.

An additional factor in the increased agglomeration in the shaker bath could be the reciprocating motion causing more frequent and violent contact between the droplets.

FIGURE 8. EFFECT OF CAPSULE SIZE ON RELEASE RATE

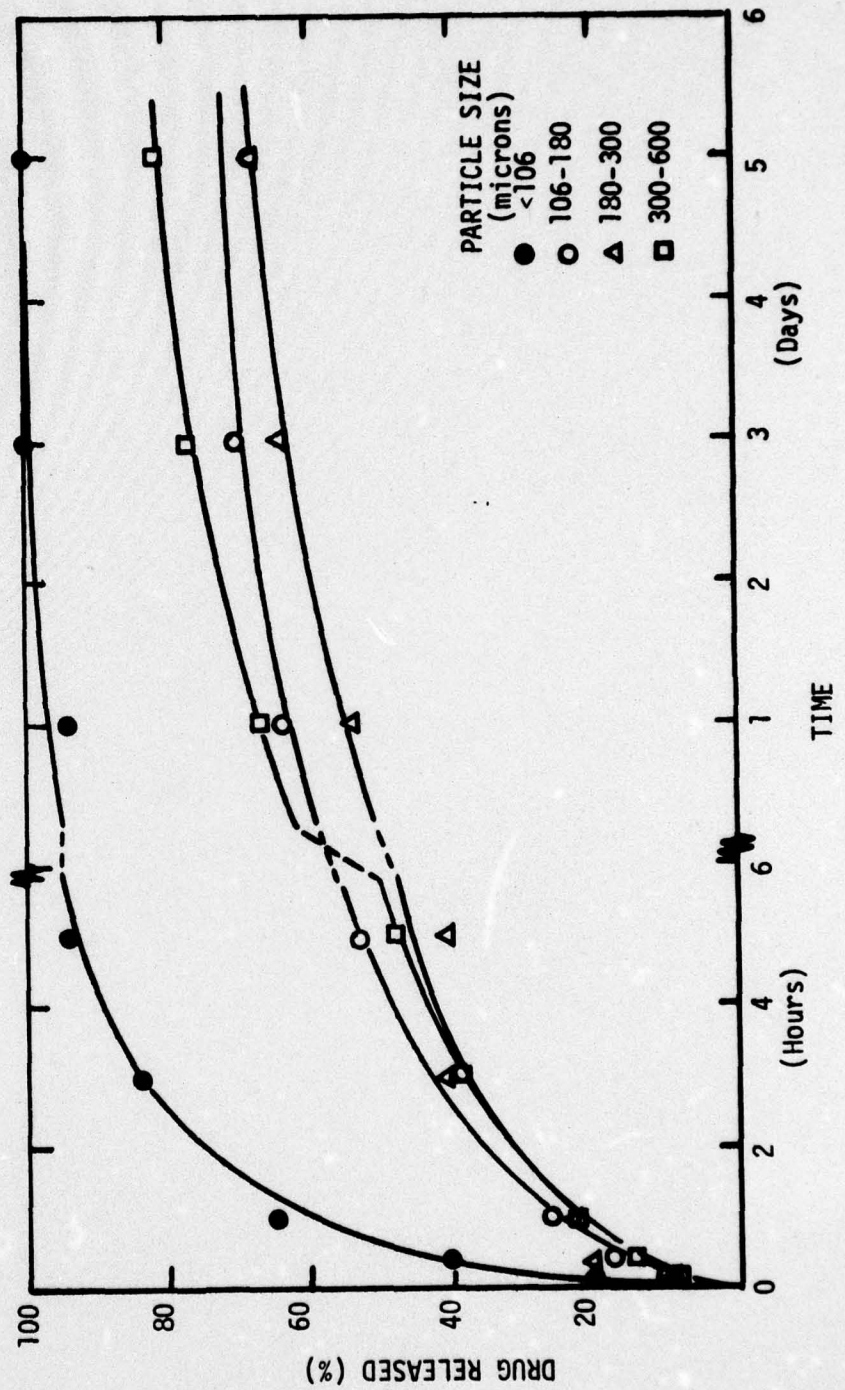
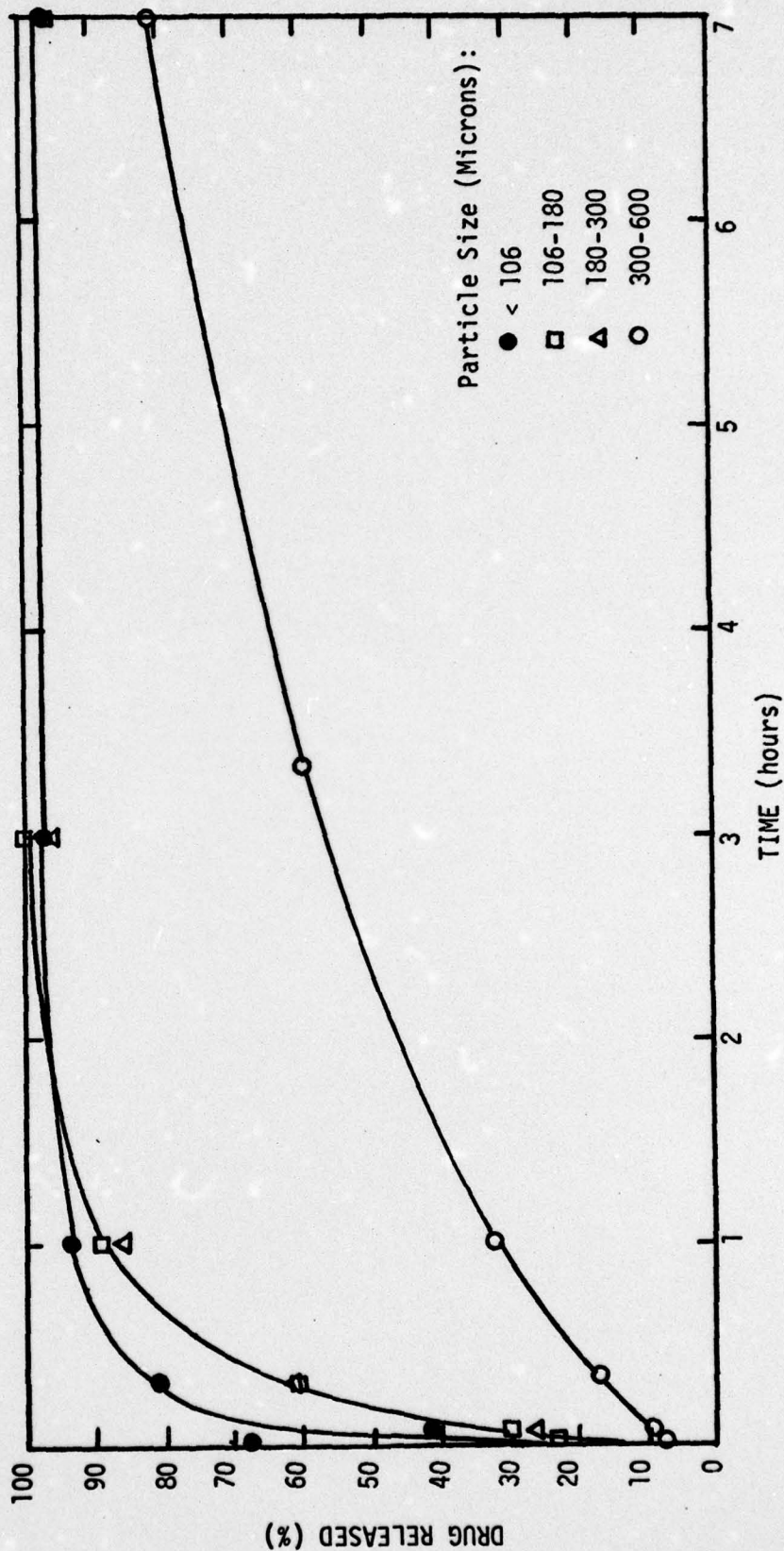


FIGURE 9. EFFECT OF MICROCAPSULE SIZE ON DRUG RELEASE



Microencapsulation of Drugs other than Astiban Acid

A few microencapsulation runs were made with antischistosomal drugs other than the Astiban acid. These were: antimony potassium tartrate, niridazole and the salt form of Astiban. The main goal of these runs was to observe whether the necessary wetting of the drug by the coacervate would take place. It did.

Figures 10,11, and 12 are photomicrographs of the microcapsules obtained in these runs. The Astiban salt, in particular, forms capsules having a good shape. However, the solubility of the drug is so high (near 30%) that it would be quite difficult to develop a capsule which would release over many hours. This was why we chose to work with the acid form of Astiban for this project period.

Niridazole gave needle-shaped microcapsules in this first set of runs. The reason for this is not yet known.

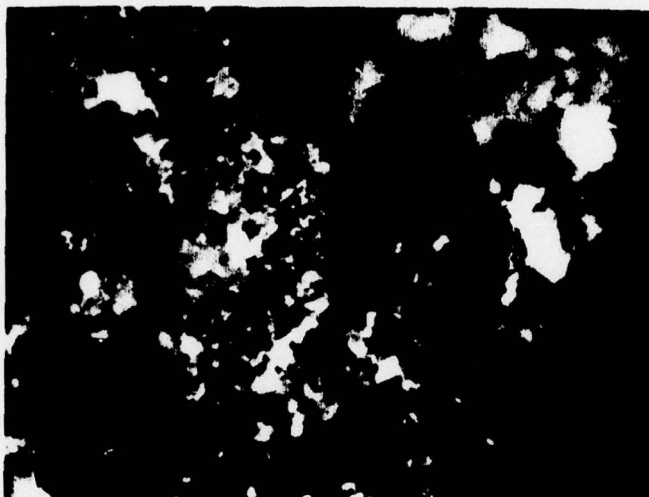
Table III shows the size distribution and the approximate half time of release of these capsules in deionized water. These represent only single microencapsulation runs. It may be seen that about 70% of the microcapsules are in the injectable range. However, the half time of release of these first capsules was too short to be practically useful. With additional work this would certainly improve, as it did with the Astiban acid capsules.

CONCLUSIONS

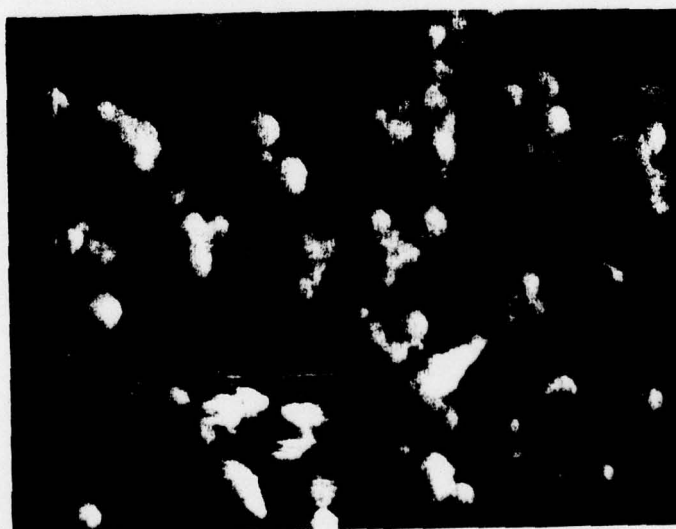
Our phase separation techniques are applicable to forming microcapsules from Astiban, Astiban acid, niridazole and antimony potassium tartrate.

In formulating a slow-release form of Astiban acid, we have now achieved the release rates shown in Table IV.

FIGURE 10. COMPARISON OF ASTIBAN
ENCAPSULATED IN POLYLACTIC
ACID WITH RAW ASTIBAN



ASTIBAN



ASTIBAN MICROCAPSULES

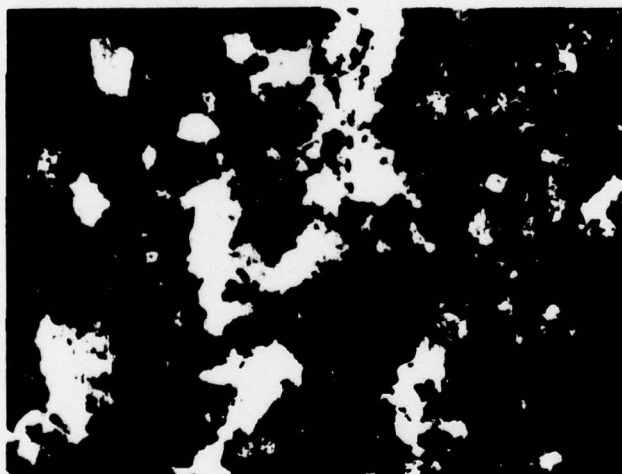
200 μm

FIGURE 11. COMPARISON OF ANTIMONY POTASSIUM TARTRATE
ENCAPSULATED IN POLYLACTIC ACID WITH RAW
ANTIMONY POTASSIUM TARTRATE



APT MICROCAPSULES
Encapsulated

200 μ m



Unencapsulated

200 μ m

FIGURE 12. COMPARISON OF NIRIDAZOLE ENCAPSULATED IN
POLYLACTIC ACID WITH RAW NIRIDAZOLE



NIRIDAZOLE IN ENCAPSULES

200μm

Encapsulated



NIRIDAZOLE

200μm

Unencapsulated

Table III. Sieve Analysis of Microcapsules

<u>Sieve Size (microns)</u>	<u>Astiban (7/1/76)</u>	<u>Niridazole (1/8/75)</u>	<u>Antimony Potassium Tartrate (11/21/74-1)</u>
> 600	-	8.9	10.2
300-600	17.0	16.4	11.2
180-300	56.0	35.5	20.7
106-180	22.0	27.9	27.9
< 106	5.0	11.3	29.5
% below 300 micron	100	74.7	77.1
Approximate half- time of release of 180-300 micron fraction	1.5 hr.	0.3 hr.	1.8 hr.

Table IV. Release Rates of Present Formulations

<u>Payload (%)</u>	<u>Release Time (hrs.)</u>	
	<u>50% Release</u>	<u>75% Release</u>
Plain Drug (Control)	0.025	0.067
41.2	30	138
55.4	16	-

In-Vivo Studies

The preliminary in vivo study of the behaviour of these microcapsules was carried out on hamsters. Five groups of hamsters, six in each group, were formed with the same average weight in each group. The first group (control) was given a subcutaneous injection of the suspending medium (peanut oil) alone. The second group was injected with nonencapsulated Astiban acid in peanut oil. The third group received nonencapsulated Astiban salt and the last two groups received two batches of microencapsulated Astiban acid.

The nonencapsulated drugs were given at 100 mg per kg and the encapsulated drug was given at 300 mgs per kg. Enough oil was used to keep the volume of each injection below 1 ml.

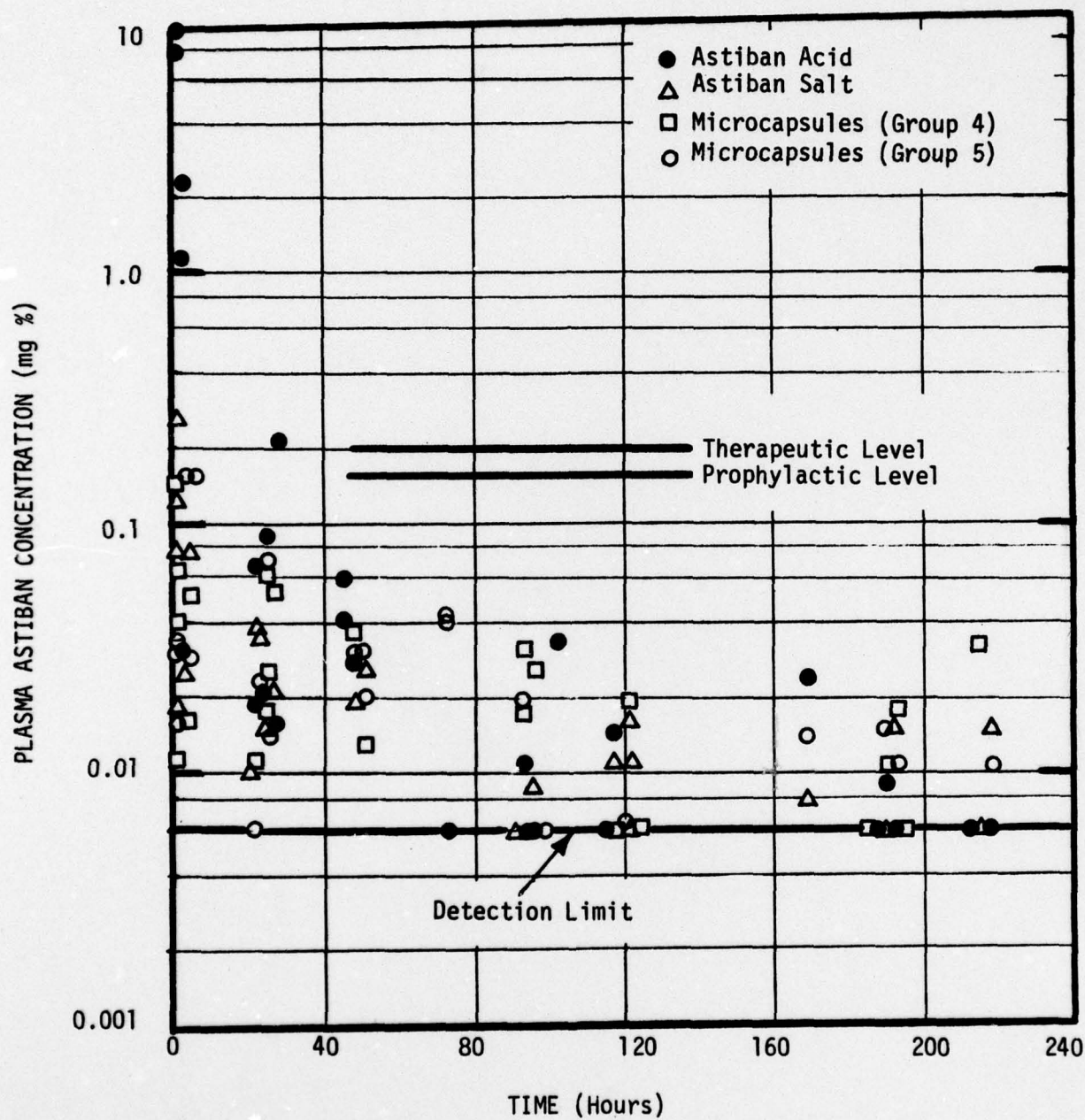
The samples of blood from the hamsters were taken at regular intervals into heparanized microsampling pipets and sealed to avoid clotting. This sample was then centrifuged in a hematocrit centrifuge to separate the plasma for analysis. The concentrations of drug in plasma are shown in Figure 13.

Observation

There were some problems in taking sufficient blood samples with the usual procedure out of the tail. Hence, the method of cardiac puncture was employed. The samples were taken for a period of about nine days. Then two healthy hamsters were sacrificed, one from each group of 4 and 5. The remaining microcapsules were recovered.

The data show no significant difference between the encapsulated and the unencapsulated drug. In both cases, minimum therapeutic blood

Figure 13.
ASTIBAN RELEASE IN HAMSTERS



levels were only maintained for a few hours. It is apparent that the drug release is not sustained in the present microcapsule formulation. From an extensive study going on in another project, after completion of this work, it has now become apparent that small amounts of residual solvent can act as a plasticizer, greatly increasing drug release rate. Conversely, release can be greatly extended using properly dried microcapsules. Future studies would aim in that direction.

REFERENCES

- Bruce, J. I. and Sadun, E. H., Prophylactic and Curative Activities of (III) Dimercaptosuccinic Acid in Olive Oil Suspension, Dicarboxylic Acid Hydrazine, An Organic Phosphorous Compound (Ronnel) and a Nitrothiazole Derivative in Rhesus Monkeys Experimentally Infected with *Schistosoma mansoni*, Am. J. Trop. Med. Hyg. 15, 324 (1966).
- Bruce, J. I. Sadun, E. H. and Schoenbecker, M. J., The Prophylactic and Curative Action of Sodium Antimony Dimercaptosuccinic Acid (TWSb) in Experimental Infections with *Schistosoma mansoni*, Am. J. Trop. Med. Hyg., 11, 25-30 (1962).
- Cicero, T. J. and C. Thies, Development of Drug Delivery Systems for Use in the Treatment of Narcotic Addiction. Quarterly Progress Reports, submitted to National Institute of Mental Health, 1974.
- Kulkarni et al, Polylactic Acid for Surgical Implants, Arch. Surg. 93, 839-843 (1966).
- Kulkarni et al, Biodegradable Poly(lactic acid) Polymers, J. Biomed. Mater. Res. 5, 169-81 (1971).
- Schneider, A.K., Polymers of High Melting Lactide, U.S. 2,703,316 (1955) assigned to duPont.
- Sparks, R. E., Controlled Release of Drugs for Treatment and Prophylaxis in Schistosomiasis and Trypanosomiasis, Proposal to U. S. Army Medical Research and Development Command, October, 1973
- Stohler, H. R. and Frey, J. R., Chemotherapy of Experimental Schistosomiasis *mansoni*: Prophylactic, Protective and Therapeutic Activity of Sodium Antimony Dimercaptosuccinate and Antimony Dimercaptosuccinic Acid in Mice and Hamsters, Ann. Trop. Med. Parasitol. 58, 280-91 (1964).
- Stohler, H. R. and Frey, J. R., Chemotherapy of Experimental Schistosomiasis *mansoni*: Influence of Dimercaptosuccinic Acid on Antischistosomal Activity of Sodium Antimony Dimercaptosuccinate and Other Antimony Compounds in Mice, Ann. Trop. Med. Parasitol. 58, 431 (1964).

-

DISTRIBUTION LIST

4 copies

HQDA (SGRD-AJ)
Fort Detrick
Frederick, MD. 21701

12 copies

Defense Documentation Center (DDC)
ATTN: DDC-TCA
Cameron Station
Alexandria, Virginia 22314

1 copy

Dean
School of Medicine
Uniformed Services University of the
Health Sciences
4301 Jones Bridge Road
Bethesda, Maryland 20014

1 copy

Superintendent
Academy of Health Sciences, US Army
ATTN: AHS-COM
Fort Sam Houston, Texas 78234